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BIOLOGICAL BULLETIN.

FORM REGULATION IN CERIANTHUS, II.

The Effect of Position, Size and Other Factors upon Regeneration.—(*Continued.*)

C. M. CHILD.

II. Discussion of Results.

The Factor of Position.

In the experiments described above, which were selected from a large number, the effect of position on regeneration has appeared in every case. Considering first the effect of position on the rapidity of regeneration, we find that in each series the rapidity of regeneration is dependent on the position which the piece occupied in the parent body, a decrease in the rapidity of regeneration occurring with increasing distance from the oral end. This relation holds for the aboral ends of pieces as well as for the oral ends, though the regenerative phenomena at the aboral ends are in most cases less sharply defined than at the oral ends. Further analysis is possible in such series as 54 and 55. The oral ends of the two sets of pieces 54*A* and 55*A* are situated at approximately the same level of the body, but the aboral ends of the pieces 54*A* are much nearer the aboral end of the parent body than those of 55*A*. The data given above for this series show very clearly that the rapidity of regeneration at the oral ends of both sets of pieces was equal, except in the final stages, while at the aboral ends it was much greater in the shorter pieces, *i. e.*, those in which the aboral cut surface was near the oral end of the parent body. From these experiments we may conclude that the two ends of a piece regenerate independently of each other, at least to a certain extent, the result depending in each case on the level which a particular cut surface occupied in the parent body.

Near the aboral end the regenerative capacity is reduced nearly

or quite to zero, cut surfaces at this level showing no regeneration beyond the union of ectoderm and entoderm over the cut muscular layer.

The influence of temperature in determining the level at which the regenerative power is reduced to zero will be discussed in another section. As regards the amount of regeneration, the effect of position is similar to its effect on rapidity. Not only is there a decrease in the rapidity of regeneration toward the aboral ends, but the total amount of oral or aboral regeneration also decreases in the same manner. In certain cases, as in Series 54, 55 and 35, the difference in size of pieces may counteract the effect of position in later stages of regeneration, so that short pieces show slightly less oral regeneration than long pieces with oral ends at the same level (Series 54*A* and 55*A*), or the amount of oral regeneration on long aboral pieces may equal that on much shorter pieces from a region nearer the oral end (Series 35, *A* and *B*). But aside from exceptions of this kind the rapidity and the amount of regeneration depend upon the level in the parent body from which the cut surface is taken, both being greatest at the oral end and decreasing aborally.

The existence of a difference in regenerative power at different levels of the body being established, the question as to its nature and significance next requires consideration. I believe that it may be due in part to a difference in the general reactive capacity of the tissues in the different regions, *i. e.*, to a decrease in the reactive power of the tissues with increasing distance from the oral end. That such a difference does exist is indicated by various facts in the normal anatomy and physiology of the animal. We must consider first the anatomical features which bear upon this point. There is a marked decrease in the thickness of the body-wall and especially of the muscular layer, toward the aboral end; the number of mesenteries also decreases toward the aboral end, until only a single pair remains; all new mesenteries appear first at the oral end and extend gradually aborally, thus indicating that growth in circumference begins orally. The physiological differences of different regions which have been noted are: the greater sensitiveness of the oral region to tactile and other stimuli, and the greater contractility of the muscles of this region when stimulated.

Since I am convinced that the power of regeneration is due, not to any special regulatory mechanism, but to those properties of organized matter which cause "normal" or "typical" growth and differentiation, I believe that the differences in regenerative power and the differences in normal anatomical and physiological reactive capacity must all depend, at least in some degree, on the general reactive capacity of the tissues.

The differences in reactive capacity in different regions may be due to differences in the intensity of metabolic change, or they may depend upon other conditions. It is of interest to note in this connection that the differentiation and concentration of nervous tissue is greatest about the oral end in the actinians. It is at least not impossible that this fact is correlated in some manner with those above mentioned. For the present, however, the possibility of such a correlation is merely suggested as the data are still insufficient for positive conclusions.

There is also a possibility that the difference in the rapidity and amount of regeneration at different levels may be due in part to differences in internal pressure resulting from circulatory currents in the enteron. Discussion of this phase of the problem is, however, reserved for a future time.

The Factor of Size.

My observations along this line are to a large extent merely incidental, since, owing to the relatively slight influence of this factor, its effects were not clearly recognized until it was too late to complete the series of experiments necessary for further study. Certain conclusions may, however, be drawn from my work and these are briefly mentioned here.

It is evident from Series 54 and 55 that great differences in size of pieces do not affect the rapidity of regeneration in earlier stages. The pieces 54*A* and 55*A*, although widely different in size, regenerate orally with equal rapidity except in the final stages. Aborally the smaller piece regenerates more rapidly than the larger because its aboral end lies in a region of greater cellular activity. As regards the total amount of regeneration at both ends the small piece exceeds the large piece, because of its position in the parent body. The only effect of size was noted

at the final stage of the experiment when it was seen that the smaller pieces were falling behind the larger pieces with respect to size of the regenerated structures. In Series 35 the possible effect of size upon the final result was noted, but here also there was no visible effect during the earlier stages.

The results afforded by these two series are confirmed by a large number of experiments. In no case, when pieces were above a certain minimal size, could any effect of size upon rapidity of regeneration be observed during the earlier stages. In the later stages it was found that regeneration became less rapid and ceased in the smaller pieces earlier than in the larger. Even in this respect differences were slight in pieces of widely different size.

The amount of regeneration is then not proportional to the size of the piece. As regards both oral and aboral cut surfaces smaller pieces show a relatively much greater amount of regeneration than larger pieces ending at the same level. In a piece one tenth the length of the body the tentacles regenerate at first with the same rapidity as in a piece nine times as long. Only after the tentacles are well formed and several millimeters in length does a difference appear. In many pieces so short that they appeared to consist only of the regenerated disc and tentacles these organs were of the same size up to a late stage as in pieces many times as large.

As regards the nature of the effect of size in regeneration in *Cerianthus* and in many other forms a few points which have suggested themselves to me may be discussed in the hope of aiding in the analysis of this phase of the problem. It may be stated as a general rule that as regeneration advances the stimulus to regeneration diminishes. We may suppose that a certain amount of material or energy is necessary for the production of regenerated structures of a certain size. This energy is derived from the substance of the body in cases like the present where no food is supplied. The smaller pieces possess of course a smaller amount of material or energy available for regeneration. This is sufficient, however, for the earlier stages, but as the amount diminishes it is probably given up less readily by the other tissues. The well-known regulative power of animal tis-

sues during starvation supports this view. Now as regeneration advances it is clear that in the smaller pieces the point where the stimulus to regeneration is no longer sufficient to cause the transfer of material or energy from the other tissues, would be reached earlier than in larger where the total supply of available material is much greater. Consequently regeneration in the smaller piece is retarded earlier than in the larger, even though the stimulus may be as great. The small piece is not necessarily exhausted, for if a new cut is made it may regenerate again, but the reserve supplies are held so closely by the other tissues that the decreasing regenerative stimulus is insufficient to render them available. A new cut surface means an increased stimulus, and under these conditions material which was not available during the later stages of the preceding regeneration may now be made available by the increased stimulus, though in this second regeneration the supply will run out sooner than before.

In the larger pieces of *Cerianthus* it is probable that the stimulus to regeneration ceases, or reaches the level of normal reparative stimuli, before a shortage of material occurs. A convenient designation for the condition of the smaller pieces is "relative exhaustion." They are not absolutely exhausted, since they may regenerate further under sufficiently powerful stimulus; they are, however, exhausted so far as the stimulus to which they are subjected is concerned. There is nothing new in the idea of relative exhaustion; it is a well-known phenomenon in biology and its rôle in regulative phenomena is undoubtedly important.

It has been impossible to determine with any degree of exactness the minimal size of pieces in which regeneration is possible. In small pieces the inrolling of the cut margins becomes so irregular that the piece often takes a form in which normal regeneration is impossible simply because of the relative position of parts. Moreover, since the regenerative power is different at different levels the minimal size of pieces differs according to the region of the body from which they are taken. Short pieces in the œsophageal region are not available for the aboral cut margin of the œsophagus unites with the aboral cut margin of the body-

wall, thus producing a condition in which the œsophagus opens aborally as well as orally to the exterior and the enteron is completely closed. As will be shown at another time, regeneration in such pieces is always slight.

The smallest pieces in which normal regeneration has been observed were about one twentieth of the body-length and from the region just posterior to the œsophagus. When the regeneration was complete these pieces appeared to consist of little but tentacles and disc, which were only slightly smaller than in pieces many times as large. My observations prove that typical regeneration is possible in very small portions of the body, provided they represent complete transverse or oblique sections of the body; pieces in which the transverse continuity is interrupted roll up in such manner that typical regeneration can never occur.

The Effect of Temperature on Regeneration.

No special experiments in relation to temperature were performed, but my work upon *Cerianthus* extended from September, 1902, to February, 1903. In September the temperature of the water was very high; during October, as the weather became cooler, it fell gradually and continued to do so as long as observed, *i. e.*, until February. A comparison of similar series of pieces begun at different times during this period affords an interesting illustration of the effect of temperature. The various series for which data are given in this paper will serve for this purpose.]

Series 22 was begun September 24; Series 35, October 20; Series 45, November 7; Series 54, 55 and 56, December 15. If the series be considered in this order a considerable decrease in the rapidity of regeneration is noticeable. In Series 22, September 24, the marginal tentacles first appeared in the pieces *A* four days after section. In Series 54, 55, 56, December 15, the marginal tentacles in the pieces *A* from about the same level of the body as Series 22*A* appeared eleven days after section. Between these two extremes lie the other series, and in every case the later in the season the series was begun the less rapid the regeneration. It is not necessary to go over the data in detail to

illustrate this point. In general it may be stated that the rapidity of regeneration in winter during the earlier stages is only about one third of the rapidity in summer.

The amount of regeneration as well as the rapidity also differs with the season. During September and October regenerated tentacles attained a length about twice as great as in similar pieces during January and February.

The length of time during which increase of size in regenerated structures, *e. g.*, tentacles, continues does not differ widely at high and low temperatures, and since regeneration is less rapid it is evident that the total amount must decrease with the temperature.

The most interesting point in connection with the effect of temperature on regeneration is the increase in size of the region at the aboral end in which regeneration does not occur. During the earlier months of my work the only pieces which failed to regenerate were the aboral tips comprising about one eighth of the body-length. Later, as illustrated in Series 45, an aboral piece about one fifth of the body-length failed to regenerate. In December, January and February pieces comprising more than the aboral third failed to regenerate or showed only slight traces of regeneration, as in Series 56*B*. Thus the portion of the body incapable of regeneration increases as temperature decreases.

In general, the effect of temperature upon regeneration in *Cerianthus* is what might be expected, since the activities of living substance in general increase and decrease with the temperature. At high temperatures it is only the extreme aboral end which cannot regenerate typically. From this "inactive" region regenerative power increases toward the oral end. As the temperature falls the limit of "inactivity" must advance toward the oral end, and more and more of the body be included in it.

The Number of Regenerated Tentacles.

It was determined that the number of tentacles regenerated is always less than the original number, and furthermore, that it decreases as the distance between the oral end of the piece and the oral end of the body increases.

It is difficult to determine with certainty the number of tentacles in living specimens of *C. solitarius*, but in fixed material

they can readily be counted. A considerable amount of material fixed for this purpose was lost by accident when too late to replace it, so that exact data cannot be given at present. The decrease in the number of tentacles with increasing distance from the oral end is, however, always noticeable even on cursory examination of the pieces.

Another spieces was also employed for this purpose, viz: the small, whitish, undetermined species mentioned in the preceding paper. In this form the tentacles are much less numerous than in *C. solitarius*, and can usually be counted without difficulty in the living specimen. This species differs somewhat from *C. solitarius* in the extent of regenerative capacity, but the course of regeneration is the same, and since the structure is in general similar to that of the other members of the family, the results obtained from this species may be accepted as typical. In all cases the regenerated tentacles were counted only after they had attained a length of several millimeters; time being thus afforded for the establishment of the small tentacles in the growing region, which sometimes regenerate somewhat later than the others. Only marginal tentacles were counted since the labial tentacles are fewer in number than the intermesenterial chambers and regenerate less regularly than the marginal tentacles. The following series are given as examples:

Series 2, October 7. — Original number of marginal tentacles 21. After removal of disc and tentacles the body was cut into five pieecs, *A*, *B*, *C*, *D*, *E* (Fig. 8). Number of marginal tentacles regenerated *A*, 0, died; *B*, 15; *C*, 14; *D*, 13; *E*, 0, did not regenerate.

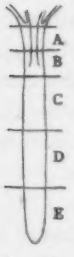

FIG. 8.

Series 5, October 19. — Original number of marginal tentacles 19. After removal of disc and tentacles body cut into five pieces, *A*, *B*, *C*, *D*, *E* (Fig. 9). Number of marginal tentacles regenerated: *A*, 0, died; *B*, 17; *C*, 15; *D*, 13; *E*, 0, did not regenerate.

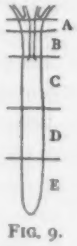

FIG. 9.

These two series are sufficient to show that the number of tentacles regenerated depends on position and has no relation to size of the piece. In both series the piece *B* is only half the size of *C*, yet it regenerates a larger number of tentacles.

These relations hold for all cases recorded, and, since the marginal tentacles correspond to the intermesenterial chambers, they are exactly what might be expected. The number of marginal tentacles which regenerate on a given piece is determined by the number of intermesenterial chambers which it possesses, or the number which it acquires by regeneration of mesenteries. The arrangement of mesenteries in *Cerianthus* was described in the preceding paper. All mesenteries appear first at the oral end and extend gradually in the aboral direction. In general, the younger a mesentery the less its length. Consequently as we pass aborally the number of mesenteries actually present decreases. After section at any level where regeneration is possible a powerful stimulus to growth exists; the mesenteries which extend into the piece remain and become united with the new œsophagus and others regenerate. It appears that the power to regenerate a given mesentery extends for a certain distance aboral to the end of that mesentery and since the mesenteries terminate at all levels we may now conclude that the "determination" of the body-wall for the regeneration of mesenteries shows a similar arrangement. Thus the youngest, shortest mesenteries can be regenerated only within a very short distance from the oral end, the next older and longer within a somewhat greater distance, and so on. The experimental results confirm and establish this conclusion in every case. The addition of new material to the body of *Cerianthus* in producing increase in circumference and number of tentacles and mesenteries may be conceived as a wedge of material which enters the body-wall at the oral end and continually forces its way aborally, thus separating the older parts of the body more and more widely. This conception of the method of growth in the transverse direction agrees with the view expressed above that the general reactive capacity of the tissues is greatest at the oral end. The stimulus to the formation of new mesenteries always becomes effective first at the oral end and from here gradually extends aborally until it is insufficient under ordinary conditions to give rise to a mesentery. Even at this level, however, a mesentery may be formed if the stimulus increases in intensity, or if the tissue becomes more susceptible to it. The resulting conditions give rise to rapid

growth and we may suppose that formative stimuli which were insufficient to produce visible results so long as the piece formed an integral part of the parent-body, either become more powerful after artificial section or else that the new embryonic tissue becomes more susceptible.

A comparative account of the factors of position, size and temperature in *C. solitarius* and the other species studied is rendered unnecessary by the close agreement between all four species. Similar results were obtained in all cases. The only points worth noting here are slight differences in the degree of regenerative power. *Cerianthus membranaceus* regenerates less rapidly than the other species. In the small whitish undetermined species the aboral region in which typical regeneration does not occur is relatively much longer than in *C. solitarius* at the same temperatures. During October only about the aboral fifth or sixth of the body is incapable of regeneration or regenerates incompletely in *C. solitarius*, while in the other species a cut surface only a short distance aboral to the middle of the body is either incapable of regeneration or regenerates incompletely. This species is much less common than *C. solitarius* and was only rarely obtained; no opportunity offered of determining its regenerative power during the colder months. Presumably, however, regeneration would have failed to occur except in the region near the oral end.

One important result of this study may be summed up in the statement that the rapidity and the amount of regeneration in pieces from the body of *Cerianthus* depend primarily on the previous relations of pieces to the parent-body, *i. e.*, on the position and consequent functional condition. This fact is of importance as indicating that the phenomena of regeneration are due not to any special regulatory mechanism which bring about return to a "normal" or "typical" form, but to the same properties which cause growth and differentiation under normal conditions.

Summary.

1. The power of regeneration from cut surfaces in *Cerianthus* is greatest at the oral end and decreases aborally, becoming null a certain distance from the aboral end. This decrease of regen-

erative power is shown by differences both in rapidity and in amount of regeneration.

2. The size of the piece does not influence the rapidity or the amount of regeneration, except in the latest stages. Since the regenerative power is different at different levels, the minimal size of a piece capable of regeneration also differs at different levels, but inversely as the regenerative power.

3. Rapidity and amount of regeneration increase and decrease with the temperature.

4. The assumption of a special regulatory mechanism is not necessary for the explanation of form regulation in *Cerianthus*.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO, July, 1903.

A STUDY OF THE RATE OF REGENERATION OF THE ARMS IN THE BRITTLE-STAR, OPHIOGLYPHA LACERTOSA.

CHARLES ZELENY.

Two interesting internal factors regulating the rate of regeneration of the arms in the brittle-star, *Ophioglypha lacertosa*, were discovered in the course of a study of the problem at the Naples Zoölogical Station during the past winter (1902–03). As some months must elapse before a full discussion of the results can be published it has been thought advisable to give the general data in a preliminary paper. This seems especially desirable at the present time because of the high interest taken in the experimental evidences of a far-reaching correlation between the parts of the individual in both animals and plants.

The experiments to be described give data which show that the rate of regeneration of the arms varies on the one hand with the size of the animal and on the other with the number of removed arms. The first-mentioned correlation gives a maximum rate of regeneration for the medium sized individuals with a pronounced decrease for the smaller as well as for the larger ones. The second correlation, with one exception to be mentioned, gives us an increase in the rate of regeneration of an arm as we pass from the cases with a smaller to those with a greater number of removed arms. The series with all five arms missing is excepted in the statement because the animals in this lot in every instance died or showed evidences of decay before the completion of the experiment.

Method. — Forty-five perfect specimens were divided into five equal groups of nine each, care being taken to distribute them in such a way as to make the sets approximately equivalent as regards size of individuals. The operation consisted in the removal of one or more arms by a transverse cut at the disk level. In the first series one arm was removed, in the second two contiguous arms, in the third three contiguous arms, in the fourth four, and in the fifth five arms. The animals were kept in ten "battery" jars, two for each series, and *were not fed during the whole period*

of the experiment. Measurements of the lengths of the regenerating arms were taken 22, 33 and 46 days after the operation. As stated above the specimens in the series where all five arms were removed did not retain their vitality for a sufficient length of time to allow of comparison with the others and they will therefore be excluded from the following tabulation.

As the rate of regeneration varies with the size of the animal as well as with the number of removed arms the proper relations can best be represented by means of curves. The figure accompanying this paper gives the average of Series I. and II. (those with respectively one and two arms removed) in one curve and of Series III. and IV. (those with respectively three and four arms removed) in the other. The same arrangement is followed for each of the three measurements taken respectively 22 days, 33 days and 46 days after the operation. This brings out the desired relations more clearly than would have been possible if all the individual data had been included. In the figure the abscissæ give the size of the animal as represented by the disk diameter in millimeters. The ordinates give the length of the regenerating arm or arms, also in millimeters. In the series where more than one arm was operated on the regeneration length as given is an average of all the regenerating arms of the individual. As the individual disk diameters are not exactly equivalent in the different series it was found convenient in taking the averages for the combination curves to use arbitrarily disk diameters equal to whole millimeters as the points for comparison. The curves in the figure are therefore constructed on this basis. The unbroken line in each case gives the average of Series I. and II. (one and two arms removed) and the broken line of Series III. and IV. (three and four arms removed).

Statement of Data and Discussion. — The curves show very distinctly the correlation between the rate of regeneration and the size of the animal on the one hand and the number of removed arms on the other.

1. Taking up first the size correlation we find that, starting with the smaller individuals, as we advance toward the larger ones there is a general increase up to a maximum at a diameter of 12 to 15 mm. This is most striking in the two later meas-

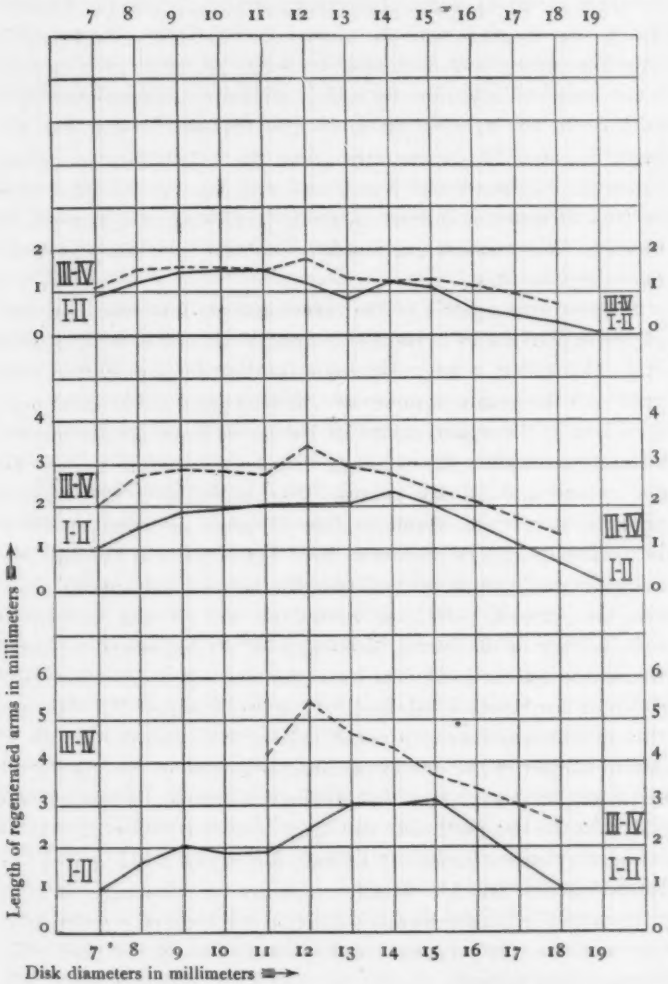

FIG. 1. The abscissæ. represent the disk diameters in millimeters and the ordinates the regenerated arm lengths, also in millimeters. The unbroken line gives the average of Series I.–II. (the specimens with one and two arms removed). The broken line gives the average of Series III.–IV. (the specimens with three and four arms removed). The uppermost curves show the conditions 22 days, the middle curves 33 days, and the lowermost curves 46 days after the operation.

urements taken 33 days and 46 days after the operation. Thus in the 33-day measurement for Series I. and II. the regenerated length increases from 1.07 mm. for a disk diameter of 7 mm. to a maximum of 2.37 mm. for a disk diameter of 14 mm. and then goes down to .21 mm. for a 19-mm. diameter. Also for the Series III. and IV. at the same time the length increases from 2.04 mm. at a diameter of 7 mm. to a maximum of 3.45 mm. at a 12-mm. diameter and down again to 1.36 mm. at a diameter of 18 mm. *The medium sized individuals thus have the maximum rate of regeneration.*

2. More striking still is the very constant difference between the average of Series I. and II. as compared with Series III. and IV. This shows a very decided advantage in favor of the animals with the greater number of removed arms. The difference is evident in the upper curves of the figure from measurements taken 22 days after the operation but becomes more striking in the 33-day and 46-day curves. For example, in the 33-day curve for a 12-mm. diameter (the diameter at which we have the maximum rate of regeneration of Series III. and IV.) we get a regenerated length of 2.08 mm. for Series I.–II. and of 3.45 mm. for Series III.–IV., an advantage of 1.37 mm. or 66 per cent. in favor of the latter. Likewise at a diameter of 14 mm. (where the Series I.–II. has its maximum regeneration) we get 2.37 mm. for Series I.–II. and 2.77 mm. for Series III.–IV., an advantage of .4 mm. or 17 per cent. in favor of Series III.–IV. In a similar manner in the curves obtained from the 46-day measurements we get at a 12-mm. disk diameter a regenerated length of 2.46 mm. for Series I.–II. and 5.42 mm. for Series III.–IV., and at a 15-mm. diameter 3.14 mm. for Series I.–II. and 3.72 mm. for Series III.–IV. which represents an advantage for the group with the greater number of removed arms of respectively 2.96 mm. (= 120 per cent.) and .58 mm. (= 18 per cent.) for the two points named.

We must therefore conclude that when more than one arm is removed the regenerative energy as expressed in the replacement of the lost arms is greatly increased. Not only is the total regenerative energy greater in this case but the energy expressed in each arm is greater than the total energy when only one is removed.

Expressing this in mathematical form, if E_1 represents the regenerative energy exhibited in the replacement of the lost arm when only one is removed, assuming that increase in length is a measure of such energy, and E_n represents the energy exhibited in regeneration when more than one arm is removed, n being the number of absent arms, then not only is $E_n > E_1$ but also $E_n/n > E_1$ or $E_n > nE_1$. Therefore when we remove n arms we increase the total regenerative energy by more than n times the amount exhibited when only one is removed. The force of this statement is made especially strong when we consider that throughout the experiments the animals received no food supply whatever.

Expressing the relation in still another way, let us take a brittle-star with arms A, B, C, D and E, in which a_1, b_1, c_1, d_1 and e_1 represent the respective lengths these arms will attain after a definite period of regeneration, supposing that one alone is cut off in each case. Now let us suppose instead that the first four are cut off, then after this same period of time we get for the regenerated lengths $a_4 > a_1$, $b_4 > b_1$, $c_4 > c_1$, $d_4 > d_1$. Now in the first case mentioned we cannot assume that the stimulus of removal and the resultant reaction of regeneration are purely local and concern only the tissues in the immediate vicinity of the cut surface for we then get into difficulty as soon as we try to explain the cases where four arms are simultaneously removed. Here we find we must add a considerable quantity (r_4) to each of the original single regeneration lengths, *e. g.*, $a_4 = a_1 + r_4$. Then $a_4 + b_4 + c_4 + d_4 = a_1 + b_1 + c_1 + d_1 + R_4$ where $R_4 (= \Sigma r_4)$ represents the total response of the organism as a whole which must be added to the local effects of the operation stimulus. If, on the other hand, we consider the influence of the organism as a whole on the regeneration of its arms as one of retardation, we must take the values a_4, b_4, c_4 and d_4 as representing most nearly the original local stimulus effect. Then without changing the values of r_4 or R_4 we may rearrange the formulæ, making $a_1 = a_4 - r_4$, etc., and $a_1 + b_1 + c_1 + d_1 = a_4 + b_4 + c_4 + d_4 - R_4$.

But whether we consider the influence of the organism as a whole to be one of acceleration or one of retardation we must recognize in either case that the regeneration rate is not a matter which involves only the local conditions at the wounded surface

as determined by the direct action of the operation. It seems, on the other hand, to be bound up with intricate reactions affecting the whole character of the activities and organization of the animal. A more direct application of the above statements to the special theories of regeneration would be out of place at the present time.

We may sum up my results on the rate of regeneration of the arms of the brittle-star, *Ophioglypha lacertosa*, as follows:

1. There is a definite relation between the size (*i. e.*, age (?)) of the animal and the rate of regeneration of its arms. The maximum rate is exhibited by individuals of medium size (with a disk diameter of 12 to 15 mm.). Both the smaller and the larger ones give a diminishing rate as we go away from this point.

2. The greater the number of removed arms (excepting the case where all are removed) the greater is the rate of regeneration of each arm.

HULL ZOÖLOGICAL LABORATORY,
THE UNIVERSITY OF CHICAGO,
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COMPARATIVE VARIABILITY OF DRONES AND WORKERS OF THE HONEY BEE.[1]

D. B. CASTEEL AND E. F. PHILLIPS.

INTRODUCTION.

According to the theory of germinal variation it would be concluded that the workers of the honey bee, *Apis mellifica*, being produced from fertilized eggs, would show more variation than would the drones which come from parthenogenetic eggs. This variation would be manifested by coloration and by relative size of parts, and it might be expected that a series of measurements made on like parts of drones and workers would show a smaller degree of variability for drones than for workers. To test this fact a series of measurements have been made and the results tabulated.

Owing to the difficulty of measuring the extent of coloration on the segments of the abdomen this could not well be used for this work, and so a series of measurements on the wings were chosen although coloration is practically the only difference usually observed between the varieties of *Apis mellifica*.

The wings are also desirable for other reasons. They are of classificatory importance in systematic work, do not shrink when preserved in alcohol and are easily examined with a microscope by simply clipping off the wing and mounting in alcohol on a slide. They also give more accurate results since the extent of coloration would vary according to the retraction of the segments of the abdomen in preserving and it would be practically impossible to get the individuals normally extended in all cases.

The reason for taking up this work was rather indirect and should perhaps be stated since the results throw some light on a widely separated line of work. Perez[2] (1878) took an Italian queen fertilized by a French black drone, and after some time examined 300 drones from this queen. As the queen was pure

[1] Contribution from the Zoölogical Laboratory of the University of Pennsylvania.

[2] Perez, J., "Mémoire sur la ponte de l'abeille reine et la théorie de Dzierzon." *Ann. Sci. Nat.*, 6 Sér., Zoöl., T. 7, 1878.

Italian her drones would also be pure Italian, since they are produced from parthenogenetic eggs, and the fact that she was mated with a black drone should make no difference. He found, however, that 149 of the drones did show some markings which he thought indicated hybridism, and from these observations rejected the theory of Dzierzon. His results were criticised severely and all manner of arguments were used against them, atavism, impurity of the queen and other reasons being given in explanation. Weighing the arguments of Perez and those presented in opposition to them, however, would lead one to believe that Perez had the best of the argument. If then we accept the theory of Dzierzon, and it is well established, we must account for the results of Perez.

An examination of a large number of hives has shown us that the coloration of the drones cannot be used as a test of their purity, and that, therefore, Perez' work is inaccurate, since he used this test as the basis of his argument. Drones from an Italian queen fertilized by an Italian drone show gradations in amount of coloration of the segments of the abdomen which would easily lead one to conclude that some of them were not pure, provided the evidence for their purity was not so strong; while at the same time the workers from the same queen show a uniformity of marking which is very striking. In the face of these facts it is evident that extent of coloration could not be used as a basis for investigation in relation to parthenogenetic development in the case of the bee. It might also be added that the fact of the irregularity of coloration of the drones is well known to most bee-keepers, and a number of these men have stated to us that they do not consider the coloration of the drones as in any sense a test of purity.

This little investigation led to the conclusion that possibly the drones showed more variation in other ways than did the workers, and to test this the measurements here recorded were made.

We wish to express our appreciation to Mr. E. L. Pratt, of Swarthmore, Pa., for material furnished, and especially to Mr. E. R. Root, of Medina, Ohio, for material and for many courtesies shown during investigations carried on by one of us in his apiary.

Measurements.

For the measurements taken in this work we have chosen veins and cells which in the bee differ from the typical hymenopterous wing and which are to a certain extent typical of the bee in their direction and extent. In this choice we have followed the discussion of the venation of the Hymenoptera of Comstock and Needham.[1] The measurements were: (1) Length of vein radius (R); (2) diagonal length of cell radius-four (R_4); (3) length of vein media-two (M_2); (4) length of medial cross-vein (m); (5) ratio between m and M_2, and (6) number of hooks or hamuli on the hind wing. An attempt was made to measure the angles formed by the union of veins radius-four (R_4) and radius-sector (Rs), and veins radius-five (R_5) and radius-sector (Rs), but on account of the difficulty of getting the exact angle at which the veins branch these measurements were discontinued through fear of inaccuracy. In all cases right wings

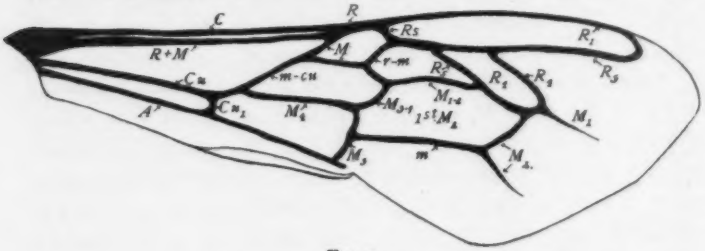

Fig. 1.

were measured. The measurements were made from camera-lucida sketches, Leitz ocular 2, objective 3 with lower lens removed and sketch made at table level, this giving a magnification of forty-two diameters. In all cases this magnification is retained in the tables.

The choice of the veins and cells measured perhaps needs some explanation since each one has certain peculiarities in direction or extent. It should be stated, however, that we do not think it makes much difference what veins are chosen for a comparison of variations in this case since all our observations show

[1] Comstock, J. H., and Needham, J. G., "The Wings of Insects," Chap. III. (continued), IX., "The Venation of the Wings of Hymenoptera." *Amer. Nat.*, Vol. 32, pp. 413–424, 12 figs., 1898.

about the same degrees of variability of the two sexes. Any other veins or cells would no doubt show like variations.

Length of Vein R. — In the typical hymenopterous wing the media (M) branches from the vein $R + M$ at a point nearer the base of the wing than in *Apis*. The length of the vein $R + M$ would therefore be desirable for measurement, but from the difficulty of getting exact measurements this was discarded, and in its place we took the measurement from the point where M branches off from $R + M$ to the point where R divides into R_1 and Rs or the length of R, which is therefore shorter than in the typical hymenopterous wing.

Diagonal Length Cell R_4. — In the typical hymenopterous wing veins R_4 and R_5 are nearly at right angles to the vein from which they branch, while in the bee, R_4 is bent out to about 135° and R_5 to 160°. This makes the cell R_4 considerably longer, and the diagonal length varies according as the angle $R_4 - Rs$ varies. The measurements were made from the proximal side of $R_5 - Rs$ to the anterior angle of $R_4 - M_1$.

Length of Veins M_2 and m. — In the bee's wing there is a bending in of the veins M_4 and M_5 toward the base of the wing with a corresponding lengthening and shifting of vein m. This vein gives a convenient measurement for the relative length of wing since it varies almost directly as the length increases. M_2 was chosen because it is correlated in its length with m, and forms a convenient relative measurement for the breadth of the wing.

Ratio between m and M_2. — As stated above, the lengths of m and M_2 are correlated in their variation, so in order to test the relative variabilities of the two veins in drones and workers, we computed the ratios between the two — $M_2 : m :: 1 : x$; x in every case being carried to two decimal places. From these computations it was found that the variation of m is in inverse proportion to that of M_2 as will be shown later.

Number of Hooks on Hind Wing. — This count was taken to see whether the hind wing varied as did the fore wing, and the number of hooks served as a conservative test.

Besides these measurements and calculations we looked for cases of abnormal wings in which the subcostal (Sc), radius-two (R_2) and cubital-two (Cu_2) veins might be present, these

being absent normally in the bee, and also for all other cases of abnormalities in the venation. In none of the wings observed were *Sc*, R_2 or Cu_2 present. The other abnormalities will be discussed later.

The Choice of Material.

The individuals used were not all taken from the same hive, since observations show that all colonies do not vary to the same extent, at least in coloration. For this reason it appeared best to use individuals from different hives and different strains in order to get a more correct idea of the natural variations. In all cases individuals were selected at random. The material used was as follows:

Drones.

I. Fifty individuals from Medina, Ohio, May 16, 1903. Hybrids, Italian and black.

II. One hundred individuals from Medina, Ohio, May 23, 1903. Italians (?) from a peculiar strain bred by F. A. Hooper, Jamaica, very light in color.

III. One hundred individuals from same hive as I. May 25, 1903.

IV. One hundred individuals from Medina, Ohio.

V. Fifty individuals from Medina, Ohio, May 9, 1903. Italians.

VI. One hundred individuals from Swarthmore, Pa., August 20, 1903. Italians from a peculiar strain bred by E. L. Pratt, the queen having an entirely yellow abdomen.

Workers.

I. Fifty individuals from same hive as Drones V., May 9, 1903.

II. Three hundred and fifty individuals from Philadelphia, Pa., August 10, 1903. Italians.

III. One hundred individuals from Philadelphia, Pa., May 15, 1902. Hybrids, Italian and black.

Length of Vein *R*.

The measurement of the length of this vein was found to be somewhat difficult owing to the hairs covering the angles and to the difficulty of getting the exact middle of the curve at the place where R_1 and *Rs* separate. However, with considerable care and

the reëxamination of cases showing the greatest variation we think the figures are nearly correct. Since the drones varied over 5 mm. on an average more than the workers and any error in measurement could scarcely be more than 1 or 2 mm. we feel justified in concluding that in this case the drones vary considerably more than the workers. The greatest variations were in Lot III. of the workers, where the variation was from 32 mm. to 42 mm., and in Lot VI. of the drones, where the variation was from 35 mm. to 48 mm. Lots I. of the workers and V. of the drones taken from the same hive at the same time show a range of variability in the ratio 8:11.

TABLE I.

VEIN *R*.

Drones.																			
Lot.	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Av.
I.					1	1	2	2	3	12	6	12	1	5	3	2				40.16
II.	1	1	1	7	8	22	19	21	11	6	3									35.99
III.					2	3	1	2	5	13	13	12	10	14	12	3	6	3	1	41.42
IV.	2	2	14	16	23	15	13	8	5	1	1									34.38
V.			1	3	3	5	11	8	7	5	3	3	1							36.92
VI.						3		9	11	18	14	10	14	8	8	3		1	1	40.42
	3	3	16	26	37	49	46	50	42	55	40	37	26	27	23	8	6	4	2	
Workers.																				
I.				2	1	3	4	11	17	9	3									37.46
II.					4	13	21	65	78	70	71	17	9		2					38.42
III.			1	1	5	11	10	26	19	12	10	2	3							37.40
			1	3	10	27	35	102	114	91	84	19	12		2					

DIAGONAL MEASUREMENT OF CELL R_4.

This measurement being taken in one of the most variable regions of the wing shows a remarkable difference in the range of variability of the two sexes. The measurement of this distance was quite easy since the limits are well marked and easily distinguishable and in no case do we think there was room for doubt in the length to more than 1 mm. This fact taken with the length makes these measurements a very good test of the relative variability. In this region of the wing a very large part of the abnormalities were found, so that we conclude that this portion shows more variation than any other; but in spite of this tendency

to vary, the workers' wings were quite constant. The greatest range of variation in workers was found to be 13 mm., while the least range in drones was 19 mm. The fact that the average length of the cell in drones was between 96 mm. and 102 mm., while the average length for workers was about 75 mm., will however account for part of this greater range since, with a given range of variability, the greater the length, the less the actual variation. This, however, does not account for the extreme difference which we find and in this case again, as in the first set of measurements, we find a much greater variability in drones than in workers.

TABLE II.

DIAGONAL CELL R_4.

Drones.

Lot.	73[1]	80[1]	87[1]	89	90	91	92	93	94	95	96	97	98	99	100
I.				1			1		2	2	2	2	3	4	6
II.				1	2	6	10	9	6	10	6	5	9	8	13
III.							1		1		1	3		6	7
IV.					7	6	5	8	5	13	13	11	8	5	7
V.									1	2	1	3	1	5	4
VI.	1	1	1			1	1	2		12	11	4	6	12	13
	1	1	1	2	9	13	18	19	15	39	34	28	27	40	50

Lot	101	102	103	104	105	106	107	108	109	110	111	112	113[1]	115	Average.
I.	2	2	3	3	4	1	5	3	3	1					101.68
II.	9	4		1			1								96.49
III.	7	8	9	9	11	10	5	8	4	6	1	1	1	1	104.14
IV.	2	4	3	1	1			1							96.15
V.	3	6	6	8	4		2	3				1			101.98
VI.	11	6	8	2	3	2	1	1	1						98.77
	34	30	29	24	23	13	14	16	8	7	1	2	1	1	

[1] No individuals found in columns omitted.

Workers.

Lot.	69	70	71	72	73	74	75	76	77	78	79	80	81	82	Average.
I.	1	1	3	6	6	12	7	3	6	3	1	1			76.36
II.	1	10	18	20	42	37	71	62	38	24	16	4	4	3	75.08
III.		1	1	3	5	8	19	20	12	12	16	2	1		76.24
	2	12	22	29	53	57	97	85	56	39	33	7	5	3	

LENGTH OF VEIN M_2.

This is not a specialized vein, and while it has for its anterior boundary the edge of the cell R_4, it shows comparatively little abnormality. A few cases of extra veins thrown in in the region of this vein will be discussed later, but it is located out-

side the portion of greatest abnormality of the wing. The principal reason for the measurement of this vein was to get a ratio with the vein *m*, but as the actual measurements add to the evidence, it seems advisable to give the table. The greatest range of variability in workers was 9 mm., the least for drones 11 mm., or, if we drop the two quite abnormal cases in Lot V., 9 mm. Taking into consideration the relative lengths of the average veins, 34 5 mm. and 45 mm., this makes the greatest variation in workers about equal to the least variation in drones, while the greatest range in drones is over twice that of the greatest range in workers.

TABLE III.

VEIN M_2.

Drones.

Lot.	32	33	34	35	36	37	38	39	40	41	42	43	44	
I.											3	2		
II.							6	8	12	6	21	16	17	
III.													1	
IV.			1							3	3	15	9	
V.								2					4	
VI.								1	1	2	1		2	
			1				6	11	13	11	28	33	33	

Lot.	45	46	47	48	49	50	51	52	53	54	55	56	Average.
I.	2		8	2	5	15	8	2	2	1			48.82
II.	6	5	2	1									42.26
III.	3	6	7	17	15	25	11	8	1	3	2	1	49.43
IV.	15	13	15	14	6	3	2	1					45.72
V.	8	5	6	10	11	2	1	1					46.96
VI.	2	3	7	11	14	13	10	15	13	2	3		48.64
	36	32	45	55	51	58	32	27	16	6	5	1	

Workers.

	32	33	34	35	36	37	38	39	40	
I.	7	12	19	9	1	2				33.82
II.	8	40	64	134	59	31	10	3	1	34.83
III.	4	10	42	22	10	11	1			34.61
	19	62	125	165	70	44	11	3	1	

LENGTH OF CROSS-VEIN *m*.

This vein shows no abnormalities and may therefore be considered as entirely outside the region so well marked about the cells R_4 and R_5, which shows so much abnormal variation. If it be argued that, since the more anterior veins tend to be abnormal,

they are not therefore good tests of the comparative variability, then, since vein *m* is entirely outside this area, this measurement would serve as an answer to that argument and is in itself a sufficient test. As in the case of vein M_2, the measurements of this vein were made principally for the sake of getting a ratio of variation between two veins which meet at nearly a right angle, but as the drones show greater variation than the workers, although not to so great a degree as in some other cases, and as this is a constant vein, we add the table. The greatest range in workers is 16 mm., the least in drones 15 mm.; the average for workers almost 13 mm., the average for drones 20 mm.; the greatest for drones 23 mm. The average lengths in the two cases (72 mm. and 97 mm.) reduces this difference about one third, so that the least variable drones are about as constant as the least variable workers, but the average range for drones still remains considerably greater than for workers.

TABLE IV.

VEIN *m*.

Drones.

Lot.	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
I.							1				1				1	3	3	1	6
II.										1			4	3	7	12	8	17	8
III.																		2	2
IV.	1		1		4	1	2	3	2	11	11	6	16	5	12	7	8	5	3
V.													2		2	3	4	2	4
VI.				1		1				1	2	2	5	5	6	10	12	14	13
	1		1	1	4	2	3	3	2	13	14	8	27	13	28	35	35	41	36

Lot.	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	Avg.
I.	1	4	2	7	5	2	3	3	3	4										100.04
II.	14	8	5	6	3	2	2													95.65
III.	4	9	5	16	4	12	8	11	10	4	2	4	2		2	1	1		1	102.43
IV.		2																		88.84
V.	9	13	1	3	2	3	1	1												97.08
VI.	6	7	7	5	2	1														94.63
	34	43	20	37	16	20	14	15	13	8	2	4	2		2	1	1		1	

Workers.

	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	Avg.
I.					1	2	2	4	6	13	7	8	4	2	1		72.30
II.	1			2	3	15	12	69	35	73	42	43	40	11	3	1	72.00
III.					1	2	1	10	16	18	27	13	8	2	2		72.43
	1			2	5	19	15	83	57	104	76	64	52	15	6	1	

Ratio Between M_2 and m.

To get this ratio the length of the vein m was divided by the length of the vein M_2 and the division carried to two decimal points giving a ratio in the form $M_2 : m :: 1 : x$. To get the comparative variability of the two sexes the individuals were tabulated according to the values of x and in this tabulation the values of x to *two* decimal points were used, but owing to the length of such a table we have combined these values and give them according to x carried to *one* decimal point. This is advisable also for another reason; owing to the relative smallness of the numbers in the first part of the proportion certain columns remained empty since, for example, no combination of figures between 32 and 57 and between 63 and 115 can give a ratio of 1:1.99. In order then to get a series of numbers which represents what is no doubt more nearly the true scale of ratios, the first table has been reduced to a table with x carried to but one decimal point. In tabulating these ratios the individuals were not grouped according to lots as in the other cases since we found practically little difference between the lots in any one sex. The average ratio for drones is 1 : 2.06 and for workers 1 : 2.08 so that no account need here be taken of the difference in averages since it is so slight. The range of the values of x for workers is from 1.70 to 2.34 or .64; that for drones from 1.68 to 2.90, or, omitting one wing with a very abnormal ratio, from 1.68 to 2.57 or .89 showing .25 greater range in drones than in workers. It might also be said, since our long table cannot be used here, that this greater range is not caused by a few abnormal cases but that the variations are shown by a gradually decreasing number of individuals in each value of x in both drones and workers, barring, of course, certain values of x which as above mentioned are impossible with the figures worked upon.

Table V.

Ratio Between M_2 and m.

Value of x.	1.7	1.8	1.9	2	2.1	2.2	2.3	2.4	2.5	2.6
Drones.	13	51	81	126	89	70	36	19	12	3
Workers.	1	11	53	119	217	81	17	1		

The working out of these ratios brought to light another point which explains this greater range in the values of x in the proportion. It was found that a certain inverse ratio exists between the lengths of m and M_2 so that the area of the cell $1st M_2$ bounded distally and posteriorly by these veins remains more nearly constant for wings of the same area than do the bounding veins. This does not mean that the area of the cell $1st M_2$ does not vary, for a little calculation shows that it does vary considerably. The ratios of the wings for each length of M_2 were gathered together and an average taken of the ratios in each case, with the result that we found a relatively constantly decreasing ratio with the increase in the length of M_2. The range of these ratios for drones was from 1.80 to 2.57, omitting again the one abnormal wing mentioned above, and for workers from 1.77 to 2.21, a difference in this case of .33 in the ranges of the two sexes. This would indicate seventy-five per cent. more range of variation for the drones than for the workers and this difference is directly correlated with the great difference in the size of M_2 found to exist.

TABLE VI.

RATIOS FOR LENGTHS OF VEIN M_2.

mm.	32	33	34	35	36	37	38	39	40	41	42	43	44	
Drones.			2.05				2.57	2.43	2.35	2.25	2.28	2.16	2.14	
Workers.	2.21	2.19	2.13	2.06	2.01	1.93	1.90	1.86	1.77					

mm.	45	46	47	48	49	50	51	52	53	54	55	56	Av.
Drones.	2.09	2.06	2.02	2.03	1.98	2.00	1.91	1.88	1.83	1.80	1.79	1.80	2.06
Workers.													2.08

HOOKS ON THE HIND WING.

The number of hamuli or hooks on the hind wing which are used to fasten the two wings together during flight were used as a means of testing the variability of this wing. The examination of 1,000 wings has shown that this is not the most variable feature of this wing, but that far more variation occurs in the breadth of the wing and in the angles of the veins which form the cross supports. As the area of the hind wing increases the increase takes place principally by a widening of the wing although by no means entirely by that method. The drones especially show

this widened wing, in some cases the width equalling the length. It may safely be stated, although no measurements were made on this point, that the area of the back wing is more variable than that of the fore wing. Since the number of hooks is correlated with the length of the wing they do not, therefore, show as much variation as would be found on the other parts of the hind wing. The least range of variation in number of hooks in workers was over seven points, the greatest, ten; the least for drones, nine, the greatest eighteen, or omitting one very abnormal wing with but twelve hooks, twelve. The relative amounts of variation are

FIG. 2.

shown far more clearly here by examining the numbers of individuals in each case which have the same number of hooks. For the workers the greatest number is 139 individuals which have 21 hooks while for drones the greatest number is 98 with 22 hooks, the average number for drones being a little higher than for workers. In workers the descent in numbers of individuals from 21 is far more sudden than that for drones, which is really

TABLE VII.

HOOKS ON HIND WING.

	12	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Avg.
Drones.																
I.				4	4	8	11	9	4	6	1	1	2			21.56
II.		2	1	12	21	23	20	17	2	2						20.12
III.				1	7	12	15	28	14	14	6	2	1			22.09
IV.			4	10	17	22	27	12	2	5	1					20.33
V.				4	2	11	10	9	7	1	4	1		1		21.54
VI.	1			3	3	7	6	23	23	19	6	6	1		1	22.42
	1	2	5	34	54	83	89	98	52	47	18	10	4	1	1	
Workers.																
I.					6	9	12	11	6	5	1					21.42
II.			4	13	42	74	94	61	45	11	4	2				21.08
III.			2	11	18	18	33	8	6	4						20.37
			6	24	66	101	139	80	57	20	5	2				

the true test of the relative variability far more than is the range of variation.

So far we have discussed the range of variability in each case, but a far more important test of the comparative variability is the relative centralization of individuals about the average point in the table. If, for example, the drones showed exactly the same range as did the workers, the fact that in every one of the tables the workers show a greater number of individuals with the average dimensions, together with a rapidly decreasing number in each direction, while the drones show a more nearly equal number of individuals over several dimensions on each side of the average point, would lead one to conclude that the drones show far more variability than do the workers. It is not necessary to go over all the tables to point out this fact, but even a hurried examina-

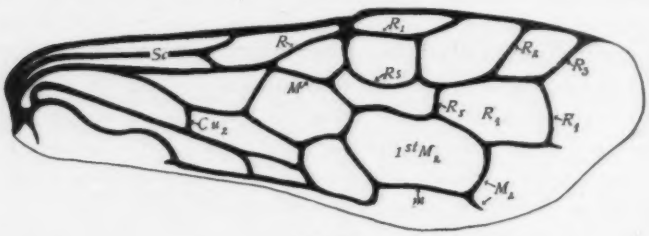

FIG. 3.

tion will show the facts as above stated. If, for example, the tables were put in the form of curves of variation the workers would show a longer and narrower curve in every case than the drones, and were these curves expressed in the form of mathematical formulæ the fact would be brought out still more strongly that the males in this case show more variation than do the abortive females.

It may be well here to offer some explanation as to why curves were not used in this work, since that is the usual method for work on variation. It was not the purpose of this investigation to work out the law of variation for the two sexes, but merely to show which sex did vary to the greater extent. This purpose has been fulfilled and is shown in the tables. In order to get a true curve of comparative variability it would be necessary to

measure many times the number of individuals which were used and to extend the observations over a far greater range of varieties. No curve made with 500 individuals would express the true law of variation, nor would ten times that number be sufficient, and since the formulation of the law of variation for parthenogenetic and fertilized forms for this particular kind of parthenogenesis, arrenotoky, is too important a matter to be based on an inaccurate mathematical formula, it seems better to us to state simply the fact that greater variation does occur in the males and leave the formulation of the law to be worked out with a far greater range of observations and measurements. And then, too, it is by no means certain that this variation follows any fixed law. If the variation were caused entirely by germinal variation, or by any other one factor, then it might be assumed that the law of this variation could be stated in the form of a mathematical formula, but as will be shown later, it appears probable that a large part of the greater variability of the drones is due to chance and is therefore not in accordance with any law. It may be argued that variation according to chance is but a way of stating our ignorance of the true law, but if there is a law for this variation it is certainly very obscure, and the working out of this law would require an extremely large number of measurements taken from individuals, each one with its life history known together with a high degree of mathematical ability in its formulation.

Abnormal Wings.

As noted previously, in all wings examined, record was made of wings having veins which are not typically found in the bee. Fig. 4 shows, in dotted lines, where these abnormalities occur most frequently. It is very difficult to record these irregularities in any kind of a table, since the irregular veins vary widely in extent and do not arise at exactly the same place in many cases. An attempt was made to classify these according to the veins from which they branch, their extent and direction. Manifestly any tabulation must be considered as merely a matter of convenience in examination. In this we have recorded cases where a vein bends (b in table) from its true course, showing but a tendency toward abnormality, as well as the well-marked cases. The ex-

tent of the abnormality is expressed roughly in the terms, very small (vs), small (s), almost complete (ac) and complete (c). The letters used to designate these abnormalities are in no way connected with the naming of the normal veins, but are chosen merely as a convenient means of marking the irregularities. It will be understood that it is impossible to draw at all times the same line between, for example, the terms small and very small,

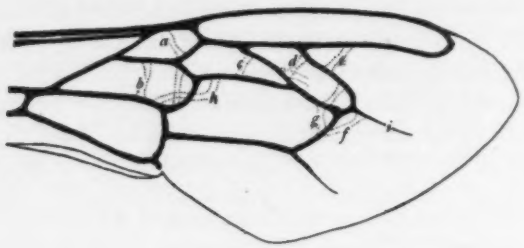

FIG. 4.

and the table is of no value except to give an idea of the number of cases of abnormalities.

	MALES.	FEMALES.
a.	82 b, 32 vs, 109 s, 1 ac, 2 irregular.	2 b.
b.	7 s, 1 double s, 4 ac, 6 c.	2 b.
c.	3 vs, 18 s, 2 ac, 13 c, 2 irregular.	1 b, 3 vs, 4 s.
d.	11 b, 27 s, 1 double s, 7 ac, 2 c.	3 b, 6 vs, 9 s.
e.	9 s, 1 ac, 5 c.	1 b, 1 vs, 3 s.
f, *g*.	8 irregular at anterior end.	2 irregular at anterior end.
h.	5 s, 2 c, 1 very irregular.	none.
i.	none.	2 lost veins; 1 almost lost.
	Other irregularities, 14.	none.

Deducting the cases where more than one irregularity occurs on one wing we have 271 irregular drone wings and 37 worker wings. Leaving out of consideration those cases in which merely a bend is recorded there are 206 irregular drone wings and 30 irregular worker wings or almost seven times as many for drones as for workers.

The figure and these tabulations make clear what has been said previously about an area of irregularity in the wing. This region is rather well defined and no irregularities in venation were seen outside it.

A point which may well be recorded is that none of these veins

could be considered as in any sense mutations since there are in all cases varying degrees of abnormality indicating a gradual variation in the directions indicated.

No attempt has been made to correlate definitely these abnormalities with any lengths of the neighboring veins, but in a general way it can be said that the largest wings of each sex are the most abnormal, and this fact explains to some extent why the drone wings are so much more abnormal than those of the workers. From this it would appear that the cause of these abnormalities was the need of extra strengthening of the cells as they became larger, and that the irregularities are the result of extra growth energy, which has a chance to show itself when room is allowed for its manifestation.

Some Explanations of These Results.

With these facts before us it seems desirable to find, if possible, some explanations for these peculiar results. As stated in the introduction the theory of germinal variation would lead one to believe that the parthenogenetic individuals of the bee would show less variation than those from fertilized eggs and if there were no complicating factors it may be supposed that this would be true, but there are certain factors which modify this result so that the ratio of variation is exactly reversed.

In the first place that kind of parthenogenesis in which males only are produced (*arrenotoky*), is not such a specialized form of agamic reproduction as are those kinds of parthenogenesis in which females or both males and females are produced. When females only result from parthenogenetic eggs (*thelytoky*), a crossing of two lines of heredity seldom occurs and amphimixis can bring about little variation. In the case of the Aphids and Daphnids we have a similar condition except that once in a year or once in some life cycle males also are produced parthenogenetically and then there is an opportunity for the blending of hereditary traits through fertilization. To this last form of parthenogenesis the name *amphoterotoky* is applied. It is thus seen that in arrenotoky this mixing of hereditary traits is not dispensed with to such an extent as in the cases of thelytoky and amphoterotoky. On the other hand, the production of females par-

thenogenetically is of far more use to a species where gamic reproduction is unnecessary, since fertilization is not necessary to give a stimulus to the egg so that it may develop, and what the species loses in lack of cross fertilization is more than made up in the advantage it has through its parthenogenetic power. Since then arrenotoky is the least specialized form of parthenogenesis, and since a crossing does occur at every *second* generation in species with this power, it follows that according to the theory of germinal variation we would find more variation in arrenotoky than in either thelytoky or amphoterotoky. In fact the decrease in variability would not be very great, since in every case but one half the crossing is dispensed with.

Variation in parthenogenetic forms has been observed previously. Weismann[1] found that the parthenogenetic ostracod, *Cyprus reptans*, showed variation, and Warren[2] found considerable variation in Daphnids. Both of these cases fall under amphoterotoky, so that on *a priori* grounds we would expect still more variation in arrentoky. It is also held by some that males tend to vary more than females, and perhaps this tendency has something to do with what we find in the case examined. Davenport and Bullard,[3] found 2½ per cent. more variation in males than in females in swine. Darwin[4] gives a considerable number of cases showing the same tendency, and others have observed similar facts. On the other hand there are cases in the Odonata (*Gomphus* and *Macrothemis*, Calvert[5]) and in the Lepidoptera (*Thyreus abbotii*, Field[6]), in which the females are more variable than the males so that we must not assume too much on this ground.

This still leaves considerable variation in drones to be accounted

[1] Weismann, A., "The Germ-plasm," 1893.

[2] Warren, E., "An Observation on Inheritance in Parthenogenesis," *P. R. Soc. Lond.*, Vol. LXV., pp. 154–8, 1899.

[3] Davenport, C. B., and Bullard, C., "Studies in Morphogenesis," VI. "A contribution to the quantitative study of correlated variation and the comparative variability of the sexes." *Proc. Am. Soc.*, Vol. 32, pp. 85–97, 1897.

[4] Darwin, Charles, "The Descent of Man." London, 1871.

[5] Calvert, P. P., "The Odonate Genus *Macrothemis* and Its Allies," *Proc. Boston Soc. Nat. Hist.*, Vol. 28, pp. 301–332, 1898. "On *Gomphus fraternus*, *externus* and *crassus* (Order Odonata)," *Entomol. News*, March, 1901.

[6] Field, W. L. W., "A Contribution to the Study of Individual Variation in the Wings of the Lepidoptera," *Proc. Am. Ac. Sc.*, Vol. 38, pp. 389–396, 1898.

for and the following facts seem to us to help in this. The workers in a hive are hatched from cells one fifth of an inch in width and the size of these cells is remarkably uniform. On the other hand the drones hatch from cells which are generally one fourth of an inch in width, but often hatch in worker cells and from cells of all intermediate sizes. In the making of the comb under natural conditions there are a great many irregular cells formed which are transition cells between the worker and drone cells, and from these, if used for brood at all, drones are produced. It is true that sometimes a worker is produced in a drone cell, but this is very rare, provided there are any worker cells in the hive. Drone pupæ, on the contrary, are frequently seen in worker cells and are very noticeable on account of the exceptionally high arched cap which the workers put on when the larva is sealed up. This then gives to drones a greater amount of variation in the room provided for their growth while in the plastic state.

A bee larva will grow until it fills the cell in which it is placed and the young bee which emerges will be the size of the cell from which it came, within certain limits. This is shown in the production of queens by the modern methods of queen rearing used in apiculture. A young worker larva, less than one day old, is lifted from its cell and put into a cell cup of queen size. The workers complete this cup and form a queen cell and the larva in this cell grows to a much larger size than would be possible if it had remained in its original cell. Once in a while the bees will attempt to make a queen from a drone larva and while, of course, this is a failure yet the result is a very large drone. Generally, however, the drone under these conditions dies before reaching the imago stage. These facts show that the growth of an individual is limited by the size of the cell and also undoubtedly by the amount of food received during the unsealed larval stage. Then it follows that since the cells from which drones hatch vary from one fifth of an inch to over one fourth of an inch in width, while those from which workers hatch are quite uniform, that the variation in size will be considerably greater for drones than for workers.

This supposition is further strengthened by some of the facts

brought out by measuring the wings. Referring again to the table of the ratios varying according to the length of vein M_2 we find that the vein *m* varies *inversely* as the length of M_2. The length of vein *m* represents the ratio of the length of the wing while vein M_2 represents the ratio of the width. Probably most of the drones which have the shortest vein M_2 are those hatched from the smallest cells and the wings could not increase in width and therefore to meet the needs of the animal in making the necessary area of wing for flight the vein *m* must be lengthened. On the other hand, those drones hatched from the largest cells would be allowed greater room for the development of vein M_2 and vein *m* need not be so long.

Another fact which seems to indicate this is that those drone pupæ which are developing in worker cells are covered over by a very high cap, making the length of their cell much greater than that of the ordinary drone cell. The drones which hatch from these cells are long and narrow when compared with those from drone cells proper.

The drones in Lot II. were taken from a hive in which there were no drone cells except possibly a very few in the corners of the frame or near the top bar of the frame since all the combs were made on what beekeepers call foundation and the cells were uniformly of worker size. These drones show the least variation since they were all hatched under the same conditions. The drones of Lots I. and III. were hatched in old irregular combs and the tables show considerably greater variability.

The greatest number of abnormalities were found on the largest drone wings and the throwing in of extra veins is probably caused by the necessity for greater strengthening of the wings. Just how these extra veins arose is not easy to explain. They may be sports, or reversions to an ancestral type, or the result of extra growth-energy or caused by the splitting of normal veins so that it is rather difficult to say just what factors bring about this extra amount of variation. While we speak of these as abnormal veins it must be noted that we do not know whether they are really abnormal or whether they are but the manifestations of a tendency possessed by all bees but which can develop only under certain conditions, just as the ovaries of the workers can develop only when extra room and food are provided.

If then germinal variation will not explain all these variations and if we accept the explanation offered as a partial and possible statement of the cause, then it would appear that the mere chance as to which cell happens to be the receptacle of a drone egg determines its variation. While it is probable that even this "chance" is according to fixed law, the fact remains that in any event this law is beyond the possibility of formulation from any observations except those extending over far more individuals than those here used. On this account we consider ourselves justified in our tabulation of results rather than in the plotting of curves and expression in mathematical formulæ, since that would be undesirable except with far more measurements and with material gathered under conditions better controlled. Our tables show the variation as it actually exists in a state of nature and the real laws can be worked out only from observations from control experiments and this it is hoped will be possible in the near future.

We do not wish to be considered as advocating the inadequacy of the theory of germinal variation to explain variation, since we have no means of knowing whether these variations can be inherited but simply wish to express the facts as we find them, and leave the explanation of the bearing of germinal variation on this problem for future investigation.

October 1, 1903.

EXPLANATIONS OF FIGURES.

FIG. 1. Fore wing of honey bee, normal. Cells and veins are named according to Comstock and Needham.

FIG. 2. Hind wing of honey bee, normal.

FIG. 3. Typical hymenopterous wing according to Comstock and Needham.

FIG. 4. Part of fore wing of honey bee showing (in dotted lines) where accessory veins were seen to occur in the wings examined. Lettering purely arbitrary as explained in text.

THE OVARIAN STRUCTURES OF THE VIVIPAROUS BLIND FISHES, LUCIFUGA AND STYGICOLA.[1]

HENRY H. LANE, A.M.

I. Introductory.

During the spring of 1902, Dr. C. H. Eigenmann collected a number of specimens of blind fishes in the caves of western Cuba, within a radius of 130 kilometers of Havana. The fishes belong to the two distinct but closely related genera, *Lucifuga* and *Stygicola*. It has been my good fortune to have the opportunity of studying the reproductive organs—more particularly, the ovarian structures—of these blind fishes, with special reference to their method of reproduction. It was discovered upon examination of the specimens that they are viviparous,[2] a fact long known in regard to some of their deep-sea relatives. Owing to the lateness of the season when they were collected, unfortunately but one female was pregnant. This one measured only 65 mm. in length and contained four fœtuses—borrowing a term to designate the post-larval stages of the young until birth—18–20 mm. long. These fœtuses were in an advanced stage of development, very probably being within a few days, or possibly hours, of birth, since a number of young only 25 mm. long were caught in the water. No other prenatal specimens having been secured, it has been impossible to study the early stages of development. My attention has been particularly directed to the ovarian structures of the mature females secured. A few of the young specimens, evidently taken not long after birth, were also examined.

I wish here to express my deep sense of obligation to Dr. C. H. Eigenmann for his assistance and criticism in the preparation of this paper.

[1] Contributions from the Zoölogical Laboratory of Indiana University, No. 58.

[2] Eigenmann, '03, p. 236, pl. 21.

II. Systematic Position of Lucifuga and Stygicola.

Poey[1] ('60) described these fishes in 1860, giving them the generic name of *Lucifuga*, recognizing them, however, as two species, *dentatus* and *subterraneus*. Later Gill, '63, separated *dentatus* from the other and created the genus *Stygicola* for it. The two genera are different in that *Stygicola* has teeth on the palatines, where *Lucifuga* has none, and the teeth in the jaws of the former are larger than those of the latter. There is also a very noticeable difference in the depth of the head at the nape, in adult individuals. The two species or genera are however so nearly alike that it is only after a prolonged comparison that the above technical differences were made out.

The several species of blind cave fishes, found in Indiana, Illinois, Kentucky and Missouri, are not related to the Cuban species. The latter are descended from marine forms which have worked their way through underground channels into the Cuban caves. Related genera that still live in the ocean about Cuba are *Brotula*[2] and *Ogilbia*[3] in moderate depths and *Bassozetus*[4] and *Aphyonus*[5] in deep water.

All of these genera belong to the family Brotulidæ, a deep-sea group comprising about forty-five genera and one hundred species, living mostly in the tropical seas of both hemispheres. The two genera under consideration in this paper are the only ones found in fresh water. Jordan and Evermann (*op. cit.*, Pt. III., p. 2498) observe very properly that "these fishes are closely related to the Zoarcidæ. In spite of various external resemblances to the Gadidæ their affinities are rather with the blennioid forms than with the latter."

III. Historical.

Numerous contributions to our knowledge of viviparity in fishes have been made from the time of Cuvier to the present.

[1] *Lucifuga*, Poey, "Memorias," II., 95, 1860 (*subterraneus*); *Lucifuga subterraneus*, Poey, "Memorias," II., 96, 1860; *Lucifuga dentatus*, Poey, "Memorias," II., 102, 1860; *Stygicola dentata*, Gill, *Proc. Ac. Nat. Sci. Phil.*, 252, 1863.

[2] *Brotula* (*vid.* Bull. 47, U. S. Nat. Mus., Jordan & Evermann, "Fishes of North America," Pt. III., p. 2500).

[3] *Ogilbia* (*vid. idem*, Pt. III., pp. 2502, 2503).

[4] *Bassozetus*, Gill (*vid. idem*, Pt. III., p. 2507).

[5] *Aphyonus*, Günther (*vid. idem*, Pt. III., p. 2525).

Among the most important ones are the following, most of which I have consulted in connection with my own investigation:

Cuvier and Valenciennes, in their "Histoire Naturelle des Poissons," I., Paris, 1828, have a short general account of viviparity in fishes, and mention is made of it frequently throughout their work in the description of such fishes as bring forth living young. Much of their work has been superseded by the more accurate observations of later investigators.

Rathké, in 1833, published his "Bildungs- und Entwickelungsgeschichte des Blennius viviparus odes des Schleimfisches." This was long the best paper on the subject.

In 1844 (*Ann. des Sc. Nat.*, t. I., 3d series, p. 313) Duvernoy published a paper on *Pœcilia surinamensis*, which is frequently referred to by more recent writers, but which I have not had the opportunity of consulting.

In 1846, Cuvier and Valenciennes described the genus *Anableps*, one species of which, *A. gronovii*, formed the subject matter of an important paper by Jeffries Wyman, in the *Boston Journal of Natural History*, Vol. VI., No. IV., p. 432, 1857. I shall refer to this article more at length below.

In 1853, Louis Agassiz (*Am. Jour. of Science*, XVI., 2d series, Nov., 1853) described a new family of fishes from California — the Embiotocidæ, which embraces the genus *Cymatogaster*. The only species of this genus, *C. aggregatus*, was studied in detail by Dr. Eigenmann, and the results published in the *Bulletin of the U. S. Fish Commission*, Vol. 12, p. 401, 1892 (1894).

Another very important paper, "On the Development of Viviparous Osseous Fishes and the Atlantic Salmon," by John A. Ryder (*Proc. U. S. Nat. Mus.*, 1885, pp. 128–162, Pls. VI.–XII.) will be noticed frequently below.

In 1887, Dr. Franz Stuhlmann made a detailed study of *Zoarces viviparus*, Cuv., the results of which he published under the title, "Zur Kenntnis des Ovariums der Aalmutter (*Zoarces viviparus*, Cuv.)." Frequent references to this volume will be made below.

IV. Viviparity in General.

Cuvier and Valenciennes (*loc. cit.*) give a general account of viviparity in fishes so far as known at that time; but since their

statements are now either matters of common knowledge or else not in accordance with the facts as revealed by later investigations, I shall not speak of their work further.

Wyman (*op. cit.*) classifies viviparous fishes into two groups "according to the position occupied by the embryo during the period of growth. In the first group may be arranged those fishes in which *the ovum leaves the ovary in an undeveloped state, and in which the process of eolution* (*sic*) *is not commenced until it reaches the lower portion of the oviduct.* The fishes which this group comprises are nearly all, if not all, Plagiostomes. The best known are *Spinax*, *Carcharias*, *Mustellus*, *Galeus*, and *Torpedo*. . . . II. In the second group those fishes are comprised in which *the gestation is wholly or in part ovarian*, the last stages only of the process usually occurring in the oviduct. Among the genera included in this division are *Silurus*, *Blennius*, *Anableps*, *Pœcilia* and *Embiotoca*. In all of these genera impregnation takes place in the ovary, and, as seems probable, while the ovum is still invested with its original envelopes."

Wyman found that each of the fœtuses in *A. gronovii* is enveloped in a separate sac of vascular tissue, much too large for the fœtus enclosed, the extra space being filled up with an albuminous fluid. He seems to regard these fœtal sacs simply as extensions of those within which the ova were suspended.

Eigenmann, considering only teleosts, found two types of viviparity (*op. cit.*, p. 404); he says:

"At least two types of viviparity may be distinguished in fishes; first, those in which the yolk furnishes all the intra-ovarian food; and second, those in which the greater part of the food is furnished by the ovary.

"In the first type the number of young is not less than in related oviparous forms, while the number of young in the second is always greatly reduced. . . . The size and development of the young in this class (type I.) of fishes at the time of birth is of course much less than in the second class of viviparous fishes."

As will be seen, *Lucifuga* and *Stygicola* belong to the second type of both Wyman and Eigenmann. The number of young is small and they are born in quite a mature condition.

V. Gross Anatomy.

For the sake of clearness the following terms will be used in the sense here given :

Oviduct — the single duct leading from the ovary to the urogenital pore. Ovisac — the forward continuation of the oviduct which covers the ovary. Ovary — the structure containing the eggs. Stroma — the supporting tissues of the ovary itself.

The term ovary is also used in a general way to include the ovisac and the ovarian structures proper. The context in every case will determine what is meant.

In *Lucifuga* and *Stygicola* the ovary is enclosed between two layers of peritoneum above the posterior portion of the alimentary canal. It may extend so far forward as to lie in part even beside the stomach.

The ovary has a bilateral arrangement. Externally it is a Y-shaped, bifurcated, subcylindrical organ, whose greatest diameter is immediately posterior to the point at which the division begins (Fig. 1). The two horns lie on the right and left sides respectively and may enclose between them the posterior portion of the stomach. Interiorly the stem of the Y is divided by a median partition with which the ovarian structures proper are associated and which extends to near the oviduct, though here only the portion attached to the ventral wall is found (Fig. 2). From the tips of the ovarian horns slender though comparatively strong threads of connective tissue, inclosing blood vessels, run forward and fasten to the peritoneal walls, thus very securely holding the ovary in position. Dorsally, the ovary is attached to the peritoneal lining of the body cavity by the mesovarium ; ventrally, there is a corresponding attachment, the mesorectum. The oviduct, which opens externally at the urogenital pore, increases gradually in size as it approaches the ovary and finally merges into the ovisac, or outer wall of the ovary.

A somewhat immature specimen shows finely those structures connected with the support of the ovary. In it one sees that each horn is supported by its own fold or lamina of peritoneum ; that these two laminæ become united at or near the point of division of the horns and are continued posteriorly as a single though thicker mesovarium supporting the body of the ovary

and the oviduct. Below there is no sign of a mesorectum in the region of the ovarian horns, except for a short distance near the base of one of them, but posterior to them there is such a membrane, inclosing several blood vessels, and itself somewhat thicker than the mesovarium above. It is also clear from an examination of this ovary that, as will be noted more particularly below, the egg-bearing tissue, the ovary proper, forms a thick median partition in the ovisac.

The external appearance of the ovary agrees very closely with Eigenmann's description (*op. cit.*, p. 418) of that of *Cymatogaster*:

"The ovary is a spindle-shaped bag, divided anteriorly into two arms which indicate the bilateral origin of the present structure. One of these arms, the left, is usually smaller than the other. . . . The ovaries of the two sides have evidently been united from behind forward, so that externally only the two anterior horns show the bilateral structure, and one of these horns seems to be in process of phylogenetic resorption."

While there is frequently a difference in the size of the two horns in *Lucifuga* and *Stygicola*, there is no uniformity in this matter. There seems to be no evidence that the right or left portion of the ovary is "in process of phylogenetic resorption." Ryder found that in *Gambusia patruelis* "the ovary is a simple unpaired organ, the greater part of which lies on the right side of the body-cavity below the air bladder. . . ."

The size of the ovary varies, of course, with the age and size of the female, as well as with the state of development of the ova or embryos contained in it. One female of the genus *Lucifuga*, which had a length of 65 mm. and which contained four fœtuses nearly ready to be born, had an ovary with a length — measuring to the extremity of the longer horn — of 16 mm., and a diameter of 8 by 9 mm. As the fœtuses were 18 to 20 mm. long, it was not surprising to find that their tails were bent over. Another female of the same genus, 83 mm. long, had evidently given birth to young only a short time before her capture and had an ovary 12 mm. in length.

The point of division into the two horns is usually about five twelfths the distance from the anterior tip of the ovary to its posterior end. As already stated, the two horns rarely show equal

PLATE I

Fig 1

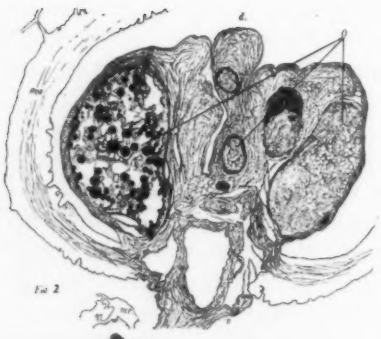

Fig 2

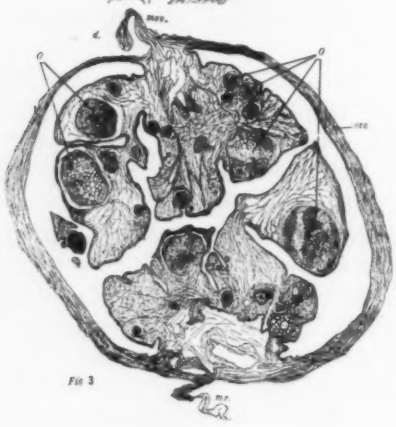

Fig 3

Fig 4

Fig 5

Fig 6

development, there being always a more or less marked difference in size.

Between the ovisac and the ovary proper there is a lumen of varying size. When there are larvæ present, the ovisac and the oviduct are extremely thin and so stretched, especially near the close of gestation, that their cellular structure cannot be made out with any satisfaction (Fig. 5). Shortly after birth of the young they contract and assume the form and appearance found in the ovaries of mature but non-pregnant females (Fig. 3). The wall of the ovisac is then quite thick, and the lumen very small. The histological structure of the ovisac will be described below.

In non-pregnant ovaries, the stroma is a mass, which, internally, has a bilateral arrangement and occupies most of the space within the ovisac (Figs. 2, 3). It is, in general shape, fusiform with its largest diameter just posterior to the division of the ovisac into the two horns down both of which it is continued along their median surfaces, forming the prongs of a Y. In the middle of the ovary the stroma forms a median partition; somewhat posteriorly this partition is cut across (Fig. 3), and still further back only the ventral part remains (Fig. 2). It has many lobes which are usually somewhat pointed and comparatively large and distinct, the indentations sometimes leaving merely a "neck" of tissue to support them. The whole stroma at this time is fully distended by the large amount of lymph contained in the sinuses described below. Where the ova are well advanced they can be seen by the unaided eye in the form of opaque dots. When the ova are surrounded by follicles, they lie some distance below the surface of the stroma and there is a tubular indentation of the epithelial covering of the latter down to the follicle (Fig. 7, *D*). In a circular space over the egg the epithelium is apparently continuous with the follicle. It is only on very close inspection that the independence of the follicle can be made out. It is then found to be of only a single cell in thickness beneath the epithelial indentation. A similar position of the epithelium was noted by Stuhlmann over the ova in the ovary of *Zoarces* and was called "Delle" by him.

The pregnant ovary is quite different in appearance from that of a non-pregnant female. A cross-section of the former shows

it to be an almost bilaterally symmetrical organ, but without any folds or pockets in which the embryos are contained as is the case in *Cymatogaster*[1] and numerous other species of Embiotocidæ. During the intra-ovarian development of the embryos, or rather the development of the fœtuses within the oviduct of *Lucifuga*, the ovarian structure proper or stroma which forms the thick median partition in non-pregnant ovaries, becomes gradually reduced and compressed into a narrow wall (Fig. 5). The stroma is much thickened both dorsally and ventrally near the oviduct (Fig. 4), where the partition is incomplete, but anteriorly its greatest thickness is near the median plane (Fig. 5). The arrangement of the stroma in each horn of the ovary is as in non-pregnant ovaries (Fig. 6, *ov.st.*).

The single oviduct runs from the caudal end of the ovary proper to open at the urogenital pore. In pregnant females it is widely distended for some distance when the fœtuses are well advanced, but in the non-pregnant females it is a rather cylindrical, thick-walled, muscular tube with numerous folds or laminæ on its inner surface, covered with a layer of columnar epithelial cells, 12 μ in depth. It is not materially different, except as to dimensions, from the ovisac described above. Stuhlmann[2] similarly found the oviduct of *Zoarces* to be a tube composed of the same cell-layers as the ovary, with the exception of the "germinal" and follicular epithelia.

VI. Histological Part.

I. The Walls of the Ovary or Ovisac.

The following system will be used to facilitate cross references to the descriptions of the various ovaries. Each ovary will be referred to by a letter, *A*, *B*, *C*, etc., the meanings of which are as follows:

A represents a female of the genus *Stygicola* with a length of 95 mm.

B represents a female of the same genus, but with a length of 128 mm.

C represents a female of the genus *Lucifuga*, length 87 mm.

[1] Eigenmann, *op. cit.*, p. 418.
[2] *Op. cit.*, p. 10.

D and *E* represent females of the same genus with a length of 83 and 65 mm. respectively. *E* was pregnant; the others were non-pregnant.

An examination of the ovisac and oviduct reveals quite a range of variation, depending in the main upon the condition of pregnancy or upon the length of time that had elapsed since the close of that period.

In the ovary of *D*, which had not been pregnant at all, or at least for so long a time that the ovarian structures had regained their normal form, the wall of the oviduct and the ovisac is from 100 to 150 μ in thickness at different places. Structurally, the ovisac consists of at least four cell-layers. The outer, a sinuated, peritoneal layer, immediatly beneath which there is a thicker layer of longitudinal muscle fibers; below this there is another somewhat thicker transverse band of muscle fibers; on the inner surface there is an epithelial layer *containing numerous capillaries.* This will be described in detail below. The nuclei of the longitudinal band are rod-like in appearance; the nuclei of the second muscle layer appear more nearly round, being evidently the cross-sections of nuclei of the same form as those in the longitudinal band. The innermost layer of epithelial cells has nuclei oval or round in shape, while the peritoneal layer shows few nuclei, but those which do appear are rod-shaped in section. Quite numerous capillaries are found between the cells of these several layers and in some places there are large blood vessels.

In the ovary *A* from a female which had evidently given birth to young but a short time previous to her capture, the ovisac measures only 15 to 20 μ in thickness. Structurally it consists of four or five thin cell-layers, between which there are anastomosing capillaries. The outermost layer consists of peritoneum, the cells of which are very much elongated and compressed. The muscle fibers beneath are mostly transverse and of the non-striated type. The inner layer is epithelial and is also much compressed. The nuclei of the muscle-fibers are long, narrow, rod-like structures which stain deeply, as would be expected, with hæmatoxylin: the nuclei of the epithelium are oval in form. The condition of this ovary does not permit me to go into greater detail.

PLATE II

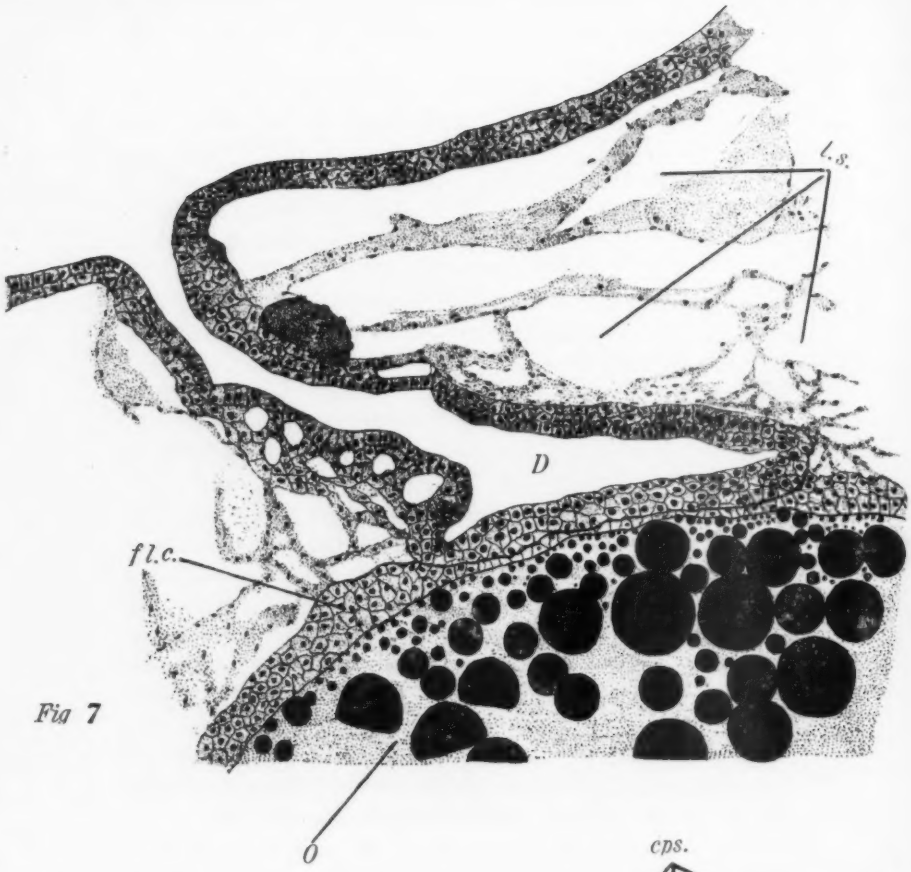

Fig 7

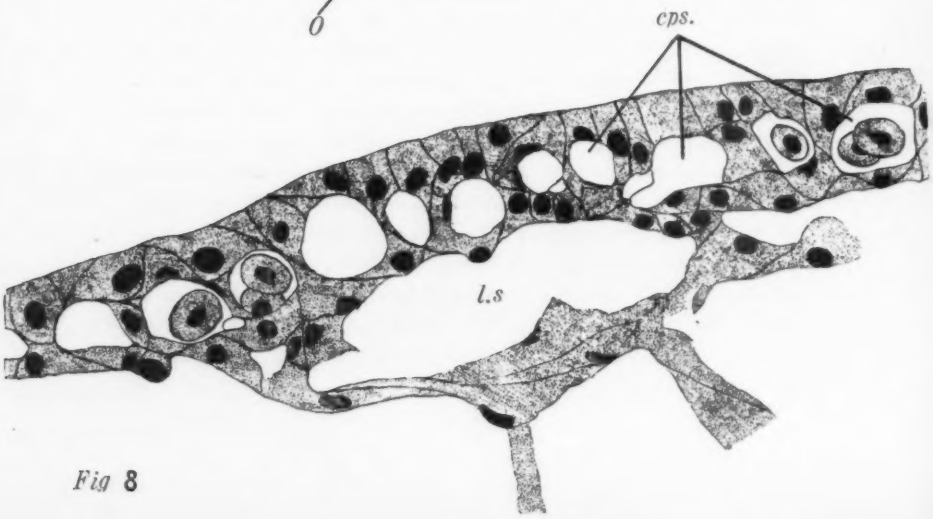

Fig 8

In the ovary of the pregnant female *E*, whose young were almost ready for birth, the ovisac is thinner than in the case just described. The different cell-layers can scarcely be distinguished, though where the cells themselves are visible, the nuclei in section have the rod-like form mentioned above. The capillaries have mostly disappeared, apparently being closed by the crowding together and stretching of the cellular structure ; but in places one comparatively large vessel appears, containing two or more rows of corpuscles, side by side. At this place the wall is enlarged somewhat to accommodate the vessel.

In the ovary of *C*, which contained ova quite well advanced, the ovisac is very similar to that described for *D*. But in some portions of this ovary the muscle layer is restricted almost entirely to longitudinal fibers, the transverse layer being much reduced. Capillaries penetrate freely through these muscle layers in all directions ; those in the lining epithelium are larger than those in the other specimens already described.

In the ovary of *B* the ovisac is similar to that in *C*, but the peritoneal covering is not so distinct ; there is the same arrangement of muscle-fibers — the outer longitudinal and the inner transverse, the latter being much the deeper. The innermost epithelial layer is composed of " pavement " cells with quite large distinct nuclei. Numerous capillaries are found in this inner lining.

Compare in this connection Eigenmann's description of the ovarian wall in *Cymatogaster* (*op. cit.*, p. 418) :

" The ovarian walls are composed, first, of the thin peritoneal membrane ; second, of a layer of longitudinal muscle fibers ; third, of a layer of circular muscle fibers, inside of which there is, in places, a layer of longitudinal fibers ; fourth, of a very thin layer of cells with flattened, deeply stainable nuclei ; fifth, of a layer of epithelium. This layer is derived from the peritoneum."

Stuhlmann found the ovarian wall in *Zoarces* to have a tolerably deep, non-striated, muscle layer, the fibers of which were closely packed together next to the peritoneal covering, but toward the lumen they were split apart by numerous sinuses containing blood vessels. The oviduct was similarly composed, except that there were few if any clefts between the fibers and there were fewer blood vessels.

The unique feature in the ovisac as well as in the epithelial covering of the stroma of *Lucifuga* and *Stygicola* is the presence of capillaries in the lining epithelium (Fig. 8). So far as could be determined this condition has never been observed in the ovary of any other form. So numerous are these capillaries that they attract attention at the first glance. The epithelium itself is often reduced to extreme thinness, sometimes serving merely as a membrane to contain the blood.

VII. The Ovary.

The ovarian structure itself is highly vascular and much lobed. There is a tendency in some instances for these lobes to be arranged in a bilaterally symmetrical pattern, when seen in cross-section, though this is not equally evident in all ovaries or even in all parts of the same ovary. The ovarian structures of the different specimens examined, while presenting numerous points in common, are yet characteristically different in every case.

The ovisac of *A* had but recently contained young, to judge from its extreme thinness; the stroma was so large that it gave promise of containing embryos. Instead of that condition, however, it was found that the large size was due to the mass of stroma which is composed in part of highly vascular tissue. Numerous blood vessels penetrate the stroma in all directions—while around the ova themselves there is a network of capillaries. The greater portion of the stroma is split up into numerous sinuses, many of which are larger than any of its blood vessels. These are closely similar in appearance to the "lymph-spaces" described by Stuhlmann (*op. cit.*, p. 19) for *Zoarces* and no doubt serve the same purpose (Fig. 7, *l.s.*).

The ova of *A* are few in number, less than ten over 60 μ in diameter appearing in any cross-section. Five or six ova are of quite large size, visible even to the naked eye, and measuring from 300 to 800 μ in diameter. They have a large amount, proportionately of yolk-substance. The smaller ova are about 50 to 60 μ in diameter, and are of the usual appearance of ova of that size. The cells of the stroma in this ovary are very irregular in shape, indistinct in outline, and usually of inconsiderable size. The nuclei are round, oval, or elongated, appar-

ently influenced as to form and shape by the cell-body. The entire surface of the stroma is covered by a layer of epithelium, with a depth of 10 to 15 μ. The nuclei of these epithelial cells appear quite distinct, are of a comparatively large size, and are round or oval in shape.

By far the largest amount of space in this epithelial layer is given up to the numerous capillaries contained in it. They are so numerous that, in cross-section, they appear as a row of large perforations, there being no more than a scant cell thickness between them. The average diameter of these capillaries is less than eight micra, in many instances being only five micra. This condition is comparable to that described above for the ovisac and is also unique (Fig. 8).

The ovarian stroma of *E*, which contained the mature fœtuses, has been squeezed and crowded into a median position by the young (Fig. 5). The cellular structure resembles that of the ovary just described, except for such variations as would be caused by its closely packed condition. The capillaries of the epithelial layer, covering its surface, are not so numerous as in the ovary of *A*, but are of larger size. The larger blood vessels are more nearly cylindrical in form and have their walls more thickened than have those in the first ovary. The lymph-spaces in this ovary are compressed by the fœtuses and temporarily eliminated.

Quite different in appearence from either of the two just described, though somewhat intermediate between them in some respects, and more advanced than the first in others, is the ovary of *D*. In this the ovarian stroma has not so many nor such large lymph-sinuses as *A*, but on the contrary has more nearly the appearance of that in *E*, from which it differs conspicuously, however, in not showing a "crowded" appearance, and in having quite numerous ova of various sizes, though none of the latter are so large as those of *A*, and in many cases are grouped together in "nests" in a way largely unknown in *A*. The blood vessels are comparatively numerous, large and quite thick walled. The capillaries in the epithelial covering of the ovary — so conspicuous is that of *A* — are so few in this case as to be visible only when carefully searched for. The cellular structure near and

next to the surface is dense and without important sinuses. The outlines of the cells are very indistinct, but the nuclei are altogether similar to those of the ovaries previously described.

The ovary of *C* approaches more nearly to the condition of *A* than has any of the others; but it differs very characteristically, since in many places it contains "nests" of ova much more conspicuous than any seen in *A*, while the largest ova in this specimen are larger than those in *A*. The ovarian structure itself, while evidently of the same character as that of *A*, does not contain quite so many lymph-spaces, and the walls of the sinuses are somewhat thicker and denser.

The ovary of *B* is almost exactly in the same stage as that of *A*. It differs from *C* in that the egg-nests have given place to single ova of considerable size and greater development than most of those in the latter.

It will be noted that the "nests" of ova are conspicuous in *Lucifuga*, though inconspicuous or lacking in the specimens of *Stygicola* examined. Whether this is a constant distinction can only be determined by the examination of more material than I have in hand.

VIII. Blood Supply to the Ovary.

A small artery, with a diameter, in different ovaries, of 20 to 75 μ, enters each horn of the ovary and runs back near the inner surface of the horn. In the main portion or body of the ovary, the two arteries occupy parallel courses near the center, separated by perhaps one third the diameter of the ovary. Since none of the specimens at hand were injected, the course of these arteries could not be traced except in a general way. But it is plain that they extend posteriorly in a tortuous course through the ovary and give off numerous branches, which find their way to or toward the surface, where they form the capillaries so distinctly visible in some of the ovaries in the epithelial covering. The blood from the epithelial capillaries of the anterior half of the ovary is collected by veinlets, frequently quite large and distinct in the vicinity of the larger ova, which join to form larger veins that pour their contents into the chief vein of the ovary at the "horseshoe bend" (infra). This largest vein has two branches

(one going out by either horn) which are united near the point of division of the ovarian horns, forming a single "horseshoe"-shaped vessel. The veinlets which return the blood from the posterior part of the ovary collect into one vessel which joins the right horn of the "horseshoe" at a considerable distance in front of the fork of the ovary, after running above and parallel to the portion with which it unites, for the distance, in one specimen at least, of nearly 2 mm.

It quite frequently occurs that red-blood corpuscles are present in the ovarian sinuses of *Stygicola* and *Lucifuga*, though their presence may be due to accident. As indicated elsewhere, these sinuses are very probably filled with a plasma or lymph.

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Explanation of Plates.

Fig. 1. External ventral view of the ovary of *Stygicola*. Portions of the peritoneal covering are visible along the sides.

Fig. 2. Cross-section of ovary near the beginning of the oviduct proper. Two large ova at the sides.

Fig. 3. Cross-section of non-pregnant ovary with stroma in two lobes — one dorsal, the other ventral.

Fig. 4. Cross-section of pregnant ovary. The section is made through a region corresponding to that of Fig. 3.

Fig. 5. Cross-section of pregnant ovary through the middle portion. The ovisac collapsed when the fœtuses were removed.

Fig. 6. Cross-section of pregnant ovary through the horns.

Fig. 7. A portion of a cross-section of a non-pregnant ovary, showing a part of a large ovum (*o*) surrounded by its follicle (*fl.c.*); the epithelial covering of the stroma dips down, forming a tube to the ovum (*D*). Bausch and Lomb one sixth objective; 2-in. ocular; tube length, 160 mm.

Fig. 8. Portion of the epithelial covering of a non-pregnant ovary showing the capillaries (*cps.*). Bausch and Lomb one twelfth objective; 1-in. ocular.

a. anterior.
cps. capillaries.
d. dorsal.
D. the "Delle."
fl.c. follicular cells.
l.s. lymph sinus.
mov. mesovarium.
mr. mesorectum.
o. ovum.
ovs. ovisac.
ov.st. ovarian stroma.
p. posterior.
v. ventral.

All drawings by the author; outlines made with Abbé camera; details put in free-hand but with the closest possible regard to accuracy.